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The endostyle of the larval (ammocoete) Lamprey, *Lampetra planeri:* a SEM study

by

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With 17 figures

Abstract

The endostyle of the larval (ammocoete) Lamprey, *Lampetra planeri,* was studied with the SEM for its anatomy and for its surface fine structure, to investigate and correlate differences in ciliary covering with the cell types of the anatomists. The present anatomical study confirms previous results concerning the spatial disposition of the chambers of the endostyle and its opening into the pharynx. The surface fine structure of the chambers of the endostyle presents several types of ciliary coverings which correspond to the different cell types described in the literature. Two grooves correspond to the zone of the secretory apices of the type I mucous cells from dorsal and ventral cylinders and one groove to the so-called formative zone. Cell types II and III are covered with dense cilia. The zone of cell type IV shows a mixed population of highly ciliated cells and cells with a nude and protruding apex. The cells of type V exhibit microvillosities and possess a single cilium which is sometimes long and curled.

Introduction

The Lamprey belongs to a present day Order of primitive Vertebrates, the Cyclostomata. The Lamprey larva, the ammocoete, exhibits an endostyle (an organ shared with Protochordates) which opens into the pharynx and which is formed of highly complex cavities. We investigated this key structure with the SEM for its spatial organization, and

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above all for its surface microstructure. This organ is also histologically very complex and five cell types are classically recognized. We tried to find if a correlation can be evidenced between surface SEM features and the histological cell types.

This endostyle (the hypopharyngeal gland of some authors), although it is a non-follicular gland, shares some biochemical features with a genuine thyroid gland: iodide uptake and organification (Gorbman & Creaser 1942; Olivereau 1955); synthesis of a glycoprotein with physical, chemical (Suzuki & Kondo 1973; Monaco *et al.* 1978) or antigenic (Wright *et al.* 1978) characteristics similar to thyroglobulin of advanced Vertebrates; synthesis and secretion of thyroid hormones, thyroxine and triiodothyronine (Leloup & Berg 1954; Leloup 1955; Roche *et al.* 1961; Clements-Merlini 1960*a*). At the onset of metamorphosis, some endostyle cells form typical thyroid follicles (Marine 1913; Leach 1939; Sterba 1953; Clements-Merlini 1960*b*; Wright & Youson 1976). Thus ontogenesis shows an endostylous phylogenetic origin of the vertebrate thyroid.

In the ammocoete endostyle, several cell types designated I to V have been described using light microscopy (Marine 1913; Leach 1939; Barrington & Franchi 1956) (fig. 1)

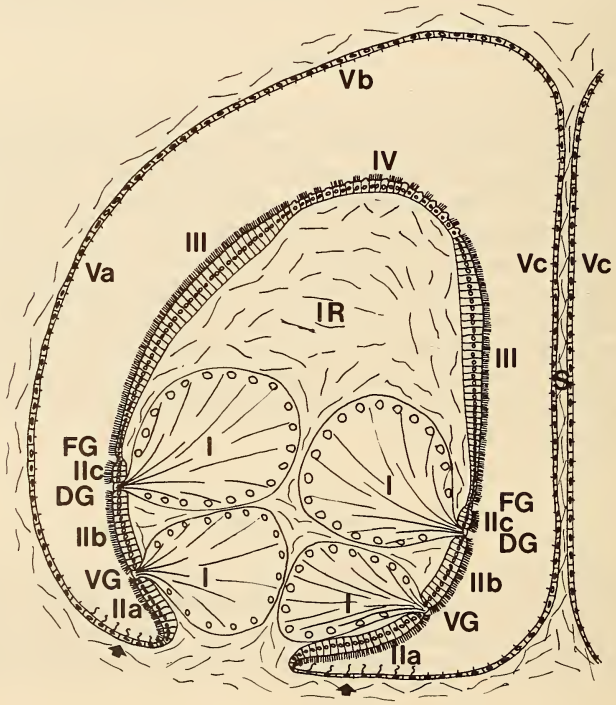


Figure 1.

Schematic transverse section of endostylar anterior chambers showing cell type localization. I-V: cell types; FG: formative zone groove; DG: dorsal groove (apices of type I cells); VG: ventral groove (apices of type I cells); arrow: transitional zone between type IIa and type V; S: septum; IR: internal rim (modified from Barrington and Franchi, 1956).

and transmission electron microscopy (Egeberg 1965; Hoheisel 1969; Fujita & Honma 1968; Wright & Youson 1980). The functions of these cell types, their ability in iodide uptake (IU) and organification and their immunoreactivity to thyroglobulin antibody (IR) are different (Gorbman & Creaser 1942; Olivereau 1955; Barrington & Franchi 1956; Clements-Merlini 1960*a*; Egeberg 1965; Fujita & Honma 1969; Wright & Youson 1976; Wright *et al.* 1978):

— Type I elongated mucous cells are localized deep in the endostyle tissue and grouped in glandular cylinders. They are IU and IR negative;
— Type II (IIa, IIb, IIc) ciliated superficial cells are either IU and IR negative (IIa and IIb) or highly IU and IR positive (IIc);
— Type III ciliated superficial cells are highly IU and IR positive and are separated from type IIc cells by a so-called formative zone;
— Type IV moderately ciliated superficial cells are IU and IR negative;
— Type V flatened superficial cells line the walls of the endostyle cavities which are sometimes subdivided into Va, Vb and Vc respectively, corresponding to lateral, dorsal and median walls; they are moderately IU and IR positive.

We have studied the fine structure of the endostyle surface to see if there were differences between the different cell types described above.

Material and methods

Larvae (6 cm long, weighing about 2.6 g) of the Lamprey, *Lampetra planeri* (Bloch 1784), coming from the estuary of the Garonne (France), were supplied by J. Ducasse and Y. Leprince who captured them in July. The animals were fixed directly in 2% glutaraldehyde in cacodylate buffer 0.05 M, pH 7.4, cut longitudinally or transversly in 2-3 mm thick slices, rinced in cacodylate buffer, dehydrated in graded ethanol, transferred to graded amyl acetate and dried in a critical point apparatus with CO_2. After gold sputtering, the endostyles were examined in a scanning electron microscope (SEM) ISI Super Mini SEM, at 25 kV.

Results

Anatomy (Figs 2-10)

Examination at low magnification of the surface of the slices of endostyle allowed us to reconstruct this organ spatially and to localize, on the transverse cuttings (Figs 4-10), the different cell types and to determine the type of ciliary covering which characterizes them. The canal which joins the endostyle and the pharynx was also checked (Fig. 9).

The endostyle is a double, symmetrical structure which is divided into two chambers by a thin wall (septum) placed in the symmetry plane of the animal. The anterior chambers are elongated and rectilinear; they each contain a voluminous rim affixed by its ventral part to the wall of the chambers (Figs 2-5). These chambers open posteriorly into the pharynx by a narrow canal (Figs 2, 3, 6, 9). Some distance before this canal, the rims spread dorsally till they come into contact with the ceiling of the chambers: they separate then the chambers into lateral and median chambers. The walls of the canal joining the endostyle and the pharynx are in continuity with the apical part of the rims (Fig. 6). Posteriorly to

the canal, the lateral chambers of the endostyle continue rectilinearly, forming little horns of narrow diameter (Fig. 8). The median chambers coil dorsally, forming a helix with two whorls (Figs 2, 3, 7). From the level of the canal backwards, in the endostyle the septum is interrupted dorsally, allowing a communication between the two median symmetrical chambers (Figs 2, 3, 6). Similarly, in the helix, the septum is incomplete and closes only halfway the communication between the two median chambers (Fig. 7). All the chambers of the endostyle (anterior chambers, posterior horns and helix) contain the prominent rim described above (particularly visible on Figs 2-7) where cell types I to IV are grouped. The cell type V lines the walls of the endostyle chambers. The cell type I (mucous cells) forms glandular masses which are elongated in longitudinal cylinders in the centre of the rims. There are four glandular cylinders in the rims of the anterior chambers (Figs 4-6), but they remain only two in the posterior horns (lateral chambers, Figs 7-8) and two in the helices (median chambers, Fig. 7). Thorough examination of the endostyle chambers allowed us to discover three grooves (arrows, Fig. 2), which run along the internal rims, from their anterior end to the centre of the helices without any interruption. Generally, the walls of the chambers (cell type V) are slightly ciliated; on the other hand, the surface of the internal rims is highly ciliated.

Surface fine structure and ciliary covering

— *Cell type I*

The cell type I (mucous cells) is grouped deep in the internal rims. However, the apices of these cells reach the surface of the rims and there discharge their mucous secretion.

Two grooves, which run along the internal rims, are associated with these discharging zones (Fig. 2). These grooves are placed between two zones of cell type II. For the dorsal glandular cylinders, the groove is relatively large and devoid of any cilium, but it seems covered by long villi, somehow glued probably by the mucous secretion discharged there (Fig. 12). A narrower groove is associated with the ventral glandular cylinder. It is generally covered by cilia of the adjacent type II cells.

— *Cell types II and III and formative zone*

Cell types IIa, IIb, IIc are localized respectively on the two sides of the grooves associated with the mucous cells cylinders on the ventral and median parts of the internal rims. A third groove (Fig. 11) runs along the internal rims, parallel and dorsal to the two other grooves. It is located at the level of the so-called formative zone, between types IIc and III and is devoid of any cilium, but covered by typical villi (Fig. 13).

We found no surface characteristics differentiating zones of cell types IIa, IIb and III. They have a dense and regular ciliary covering, the cilia of which are relatively long and almost linear (Fig. 14). However the zone of cell type IIc, located between the dorsal groove of cell type I and the groove of the formative zone, has longer and finer cilia than the other zones of this type II. Moreover, its cilia are sometimes sinuous (Fig. 12). The combined region formed by the groove associated to the dorsal glandular masses, the zone of cell type IIc and the groove of the formative zone forms a very distinct ribbon which runs along the internal rims and which is very conspicuous even at a low magnification (Fig. 2, 25×) or a relatively low magnification (Fig. 11, 1200×).

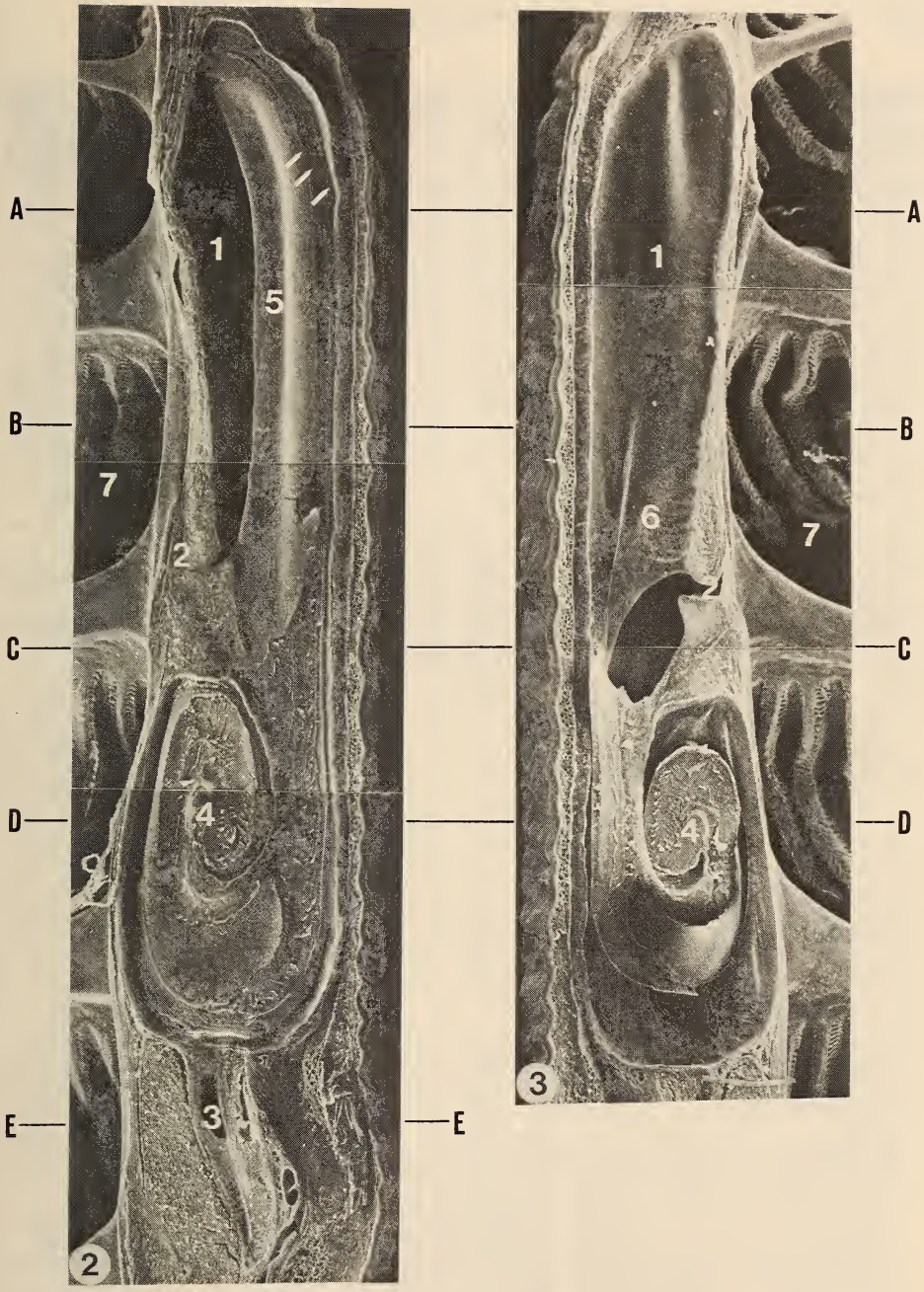


PLATE I.

FIGURE 2.

Left part of the endostyle. G = 25×.

FIGURE 3.

Right part of the endostyle. G = 25×.

A-A, B-B, C-C, D-D, E-E: section planes corresponding to Figs 4-8.
1: anterior chambers; 2: pharyngo-endostylar canal; 3: posterior horns (lateral chambers); 4: helix (median chambers); 5: internal rims; 6: septum; 7: pharynx; arrows: grooves.

PLATE II.

FIGURE 4.

Transverse section (level A-A of Figs 2-3) through anterior chambers. G = 50×.

FIGURE 5.

Transverse section (level B-B of Figs 2-3) through anterior chambers. G = 50×.

FIGURE 6.

Transverse section (level C-C of Figs 2-3) at the level of the pharyngo-endostylar canal. G = 50×.

FIGURE 7.

Transverse section (level D-D of Figs 2-3) through the helix (median posterior chambers). G = 50×.

FIGURE 8.

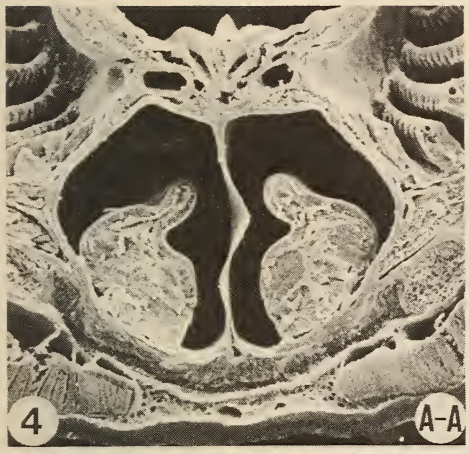
Transverse section (level E-E of Figs 2-3) through posterior horns (lateral chambers). G = 50×.

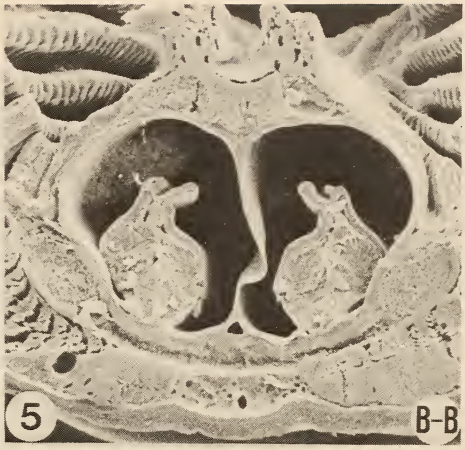
FIGURE 9.

Opening (arrow) of the pharyngo-endostylar canal into the pharynx in the median pseudobranchial groove. G = 20×.

FIGURE 10.

Transverse section of the internal rim showing the three grooves FG (formative zone groove), DG (groove associated to dorsal cylinder of mucous cells I) and VG (groove associated to ventral cylinder of mucous cells I). G = 600×.


4
A-A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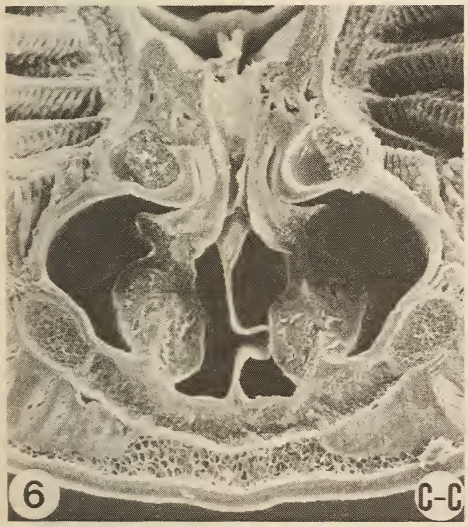
B-B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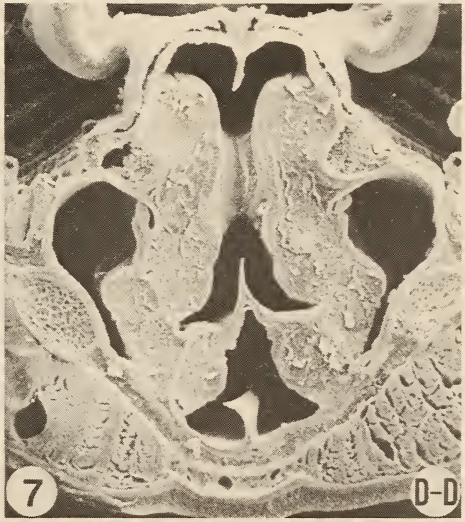
C-C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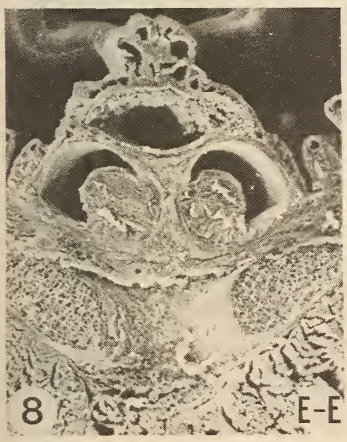
D-D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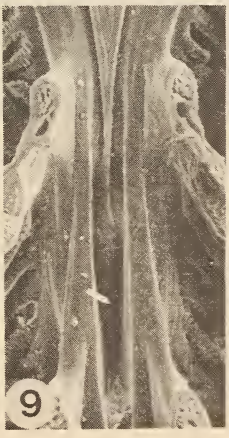
E-E


9


III
FG
IIc
DG
IIb
VG
IIa
10

PLATE III.

FIGURE 11.

Dorsal complex ribbon with cell type I apices groove (DG), formative zone groove (FG) and cell type IIb, IIc and III zones. G = 1200×.

FIGURE 12.

Dorsal groove (apices of type I cells) with glued villi between type IIb and IIc zones. G = 3000×.

FIGURE 13.

Narrower dorsal groove with villi, corresponding to the formative zone (FG). G = 6000×.

FIGURE 14.

Cell type IIb zone. G = 3000×.

FIGURE 15.

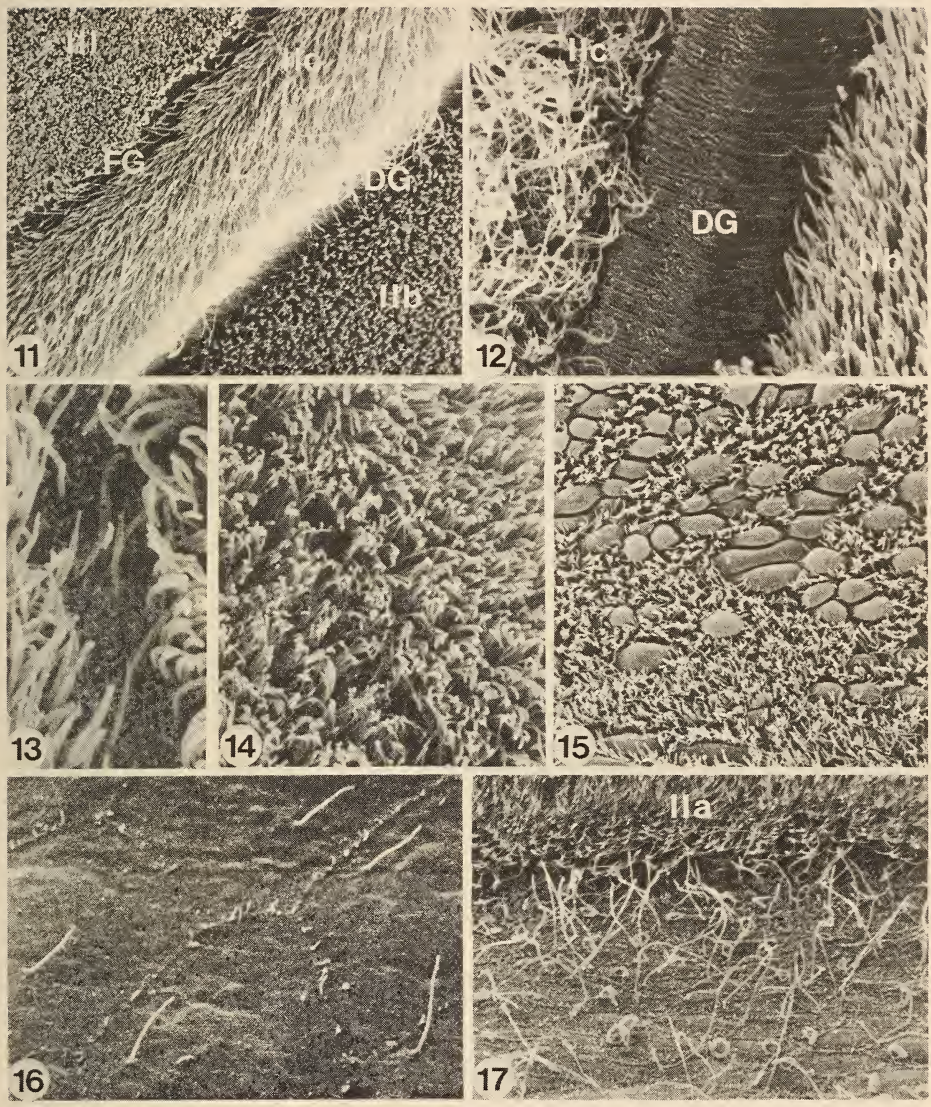
Cell type IV zone, with some nude protruding apices. G = 1200×.

FIGURE 16.

Cell type Vc zone (septum), with one cilium per cell and villi on cell borders. G = 3000×.

FIGURE 17.

Transitional zone between cell type IIa (rim) and cell type V. One long and curled cilium per cell. G = 1700×.


IIc
FG
DG
IIb
11
IIc
DG
12
13
14
15
16
IIa
17

— *Cell type IV*

The type IV zone (Fig. 15) is located on the dorsal part of the rims. Some cells, the surfaces of which are nude and the cilia of which are located only on the cell borders can be seen through the dense and regular ciliary covering which characterizes the type III zone. These nude cells ordinarily exhibit a protruding apex. They are more numerous on the very apex of the internal rims, as well as in the canal which runs between the endostyle and the pharynx; the walls of this canal are in continuity with the dorsal elongated apices of the internal rims. Near the opening of this canal into the pharynx, the ciliated cells are mixed with cells which are covered by microvilli or microridges, which are characteristics of the buccal cavity (MALLATT 1979).

— *Cell type V*

All the cells of the type V zone exhibit only one cilium. The cell surface is more or less covered by microvilli which are often limited to the cell borders. The unique cilium is generally short and linear (Fig. 16). However, on the ventral region of the endostyle chambers, between the internal rims and the walls (Fig. 1, arrows), the cells of the walls have one very long and sometimes curled cilium. Moreover, these cells are relatively rich in microvilli (Fig. 17).

DISCUSSION

The use of SEM for studying the surface of the cells which line the internal walls and rims of the endostyle allowed us to describe up to seven different types of ciliary covering which can be attributed to the different kinds of cells described in the literature (see Introduction). The correspondancies were easy to make between our surface images and those published on histological sections as concerns the cell types. However, some details, which were already perceived by the histologists, have been clarified, particularly those concerning the complex ribbon running longitudinal along the internal rims. This special region is characterized by a narrow groove related to the secretory apices of cell type I previously described (FUJITA & HONMA 1968) and by a ciliated band of cells of type IIc (whose cilia have a particular design) which is limited dorsally by a second groove. This latter groove corresponds to the formative zone (immature cells of type III), a narrow region of small cells which is generally considered as a proliferative zone of the cells of type III (CLEMENTS-MERLINI 1960*a*; BARRINGTON & SAGE 1963; BARRINGTON & FRANCHI 1956; HOHEISEL 1969; FUJITA & HONMA 1968). The type IIc cells, whose ciliary covering is different, seem to have a particularly high iodide uptake (CLEMENTS-MERLINI 1960*a*; FUJITA & HONMA 1969; BARRINGTON & FRANCHI 1956).

Concerning the zone of cell type IV, no precise limit with the zone of cell type III was discernable. Moreover, the cells seem to be of two types: the ciliated cells, which are totally comparable to the cells of the type III zone, and the cells whose apex is protruding, nude and without cilia. These differences are also visible in TEM and the cilia are seen pushed back to the periphery of the apices (EGEBERG 1965; FUJITA & HONMA 1968; WRIGHT & YOUSON 1980). The zone of cell type IV is the only zone to exhibit a mixed population of cells; apical protuberances could be the site of apocrine activity (EGEBERG 1965; FUJITA & HONMA 1968). For the zones of cell type V, BARRINGTON & FRANCHI (1956) and WRIGHT & YOUSON (1980) mention that the flat cells V become tall columnar at the place where they adjoin type IIa cells (Fig. 1, arrows). This cell modification is also expressed

by the different ciliary covering of these zones on our images (long and sometimes curled cilia). Furthermore, no author ever mentionned the presence of cilia or villi on the surface of the epithelia of type V or in the transitional zones between type V and the rims (Barrington & Franchi 1956; Wright & Youson 1980; Fujita & Honma 1968). However, with the SEM we could observe the constant presence of a unique cilium and often of microvilli at the periphery of the cellular apices on each cell of type V zones as well as in the transitional zone. The unicity of the cilium perhaps explains that no cilium was found or mentioned in TEM studies.

The dense ciliary covering of the endostylar epithelia (principally type II and III) as well as the existence of microvilli is consistent with a local circulation of water and possibly of mucus through the chambers of the endostyle (Leach 1939; Barrington 1972). However, the scarcity or absence of cilia in the canal running between the endostyle and the pharynx seems to limit the efficiency of the water flow at the entrance of the endostyle.

On the other hand, cilia and microvilli may be correlated with intense surface activities in relation to iodide organification and secretion. However, our results do not allow us to clarify the difficult question of mode of discharge of the hormonal secretion, that is output of hormones at the basement membrane, which is not accessible directly to a SEM study, or apical excretion in the endostylar chambers and possible reabsorption of a part of material by type V cells (Clements-Merlini 1960*a*; Fujita & Honma 1969; Egeberg 1965).

Finally despite many studies, there is no definitive agreement as to which cell types contribute to the formation of follicles of the adult thyroid during metamorphosis. All cell types, except type I, have been implicated in this process: type IV and some type V (Marine 1913; Leach 1939), mainly type III (Sterba 1963; Clements-Merlini 1960*b*), type IIc, III and V (Wright & Youson 1980). Furthermore, three kinds of follicular cells have been described in adult lamprey thyroid: one ciliated type and two non ciliated types (Fujita & Honma 1966). A SEM study of the endostyle during metamorphosis could provide some evidences on which cell types contribute to follicle formation. Such a study is in progress.

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